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Biodevice Technologies for Cancer Diagnosis Using Exosome-based Biomarkers

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Abstract

Recent development of biodevice technology for analyzing new cancer biomarkers such as microRNA and exosomes are introduced, and prospects of their future practical use in medical and pharmaceutical fields will be shown.

1. Introduction

To overcome cancer and other intractable diseases, we need to create a seamless, comprehensive process where the heralding signs and lesions of diseases are precisely detected by early diagnosis via screening and by accurate diagnosis using imaging techniques, highly effective and safe treatment is provided by combining surgeries as topical treatments and drug therapies as systemic treatments based on the condition of the disease, and the lesion is reconstructed after treatment. This will eventually bring innovation to the medical system, enabling secure provision of high-quality, cost-effective medicines to anyone, anytime, and anywhere. In addition, we intend to be a global pioneer in establishing a new medical industry that leads the future of the world's most aged country, Japan.

In this presentation, I will present recent development of the bioanalytical platform applicable for developing exosome-based diagnosis, which allows detection of individual nanoparticles or nanovesicles of less than 20 nm in diameter and enables the characterization of nanoparticles based on indexes such as concentration, diameter, zeta potential, and surface protein expression. Moreover, another bioanalytical platform applicable to exosomal miRNA diagnosis will be presented to show a prospect that the time required for the cancer diagnosis can be greatly shortened to less than one hour owing to the recent progress in integrated microfluidic device technology.

2. Exosome biomarkers

Extracellular vesicles (EVs) including exosomes and microvesicles have attracted considerable attention in the fields of cell biology and medicine. For a better understanding of EVs and further exploration of their applications, the development of analytical methods for biological nanovesicles is required. However, it is technologically difficult to analyze or identify a heterogeneous population of particles ranging from several tens to one hundred nanometers, and hence, there is a growing demand for a new analytical method of nanoparticles among researchers working on extracellular vesicles.

To understand biological functions of EVs, various analytical methods are used at present. For characterization of molecular composition of exosomes, the omics techniques such as microarray, sequencer, and mass spectrometry can be used [1-3]. Furthermore, for evaluation of physical properties of exosomes, nondestructive physical techniques such as electron microscopy, atomic force microscopy, optical single particle tracking [4], resistive pulse sensing [5], dynamic light scattering, flow cytometry, and particle electrophoresis are available. However, when studying the physiological and pathological roles of exosomes, it is important to remind the presence of EV subtypes with different biogenesis pathways [6]. Namely, surface protein profiling is strongly desired in characterizing EVs. Although recent improvements in high-resolution fluorescent flow cytometry have enabled the detection and analysis of fluorescence-labeled vesicles with a diameter of up to 100 nm, the lower detection limit in size is far from sufficient for exosome research and the requirement for an experienced operator and expensive apparatus will limit their use [7]. On the other hand, surface proteins of biological particles can also be evaluated by measuring their zeta potential. The electrophoresis system that uses a microcapillary chip as an electrophoresis chamber is recognized as the most advanced and reliable method for obtaining the zeta potential of heterogeneous particles like cells or EVs [8]. Recently, an on-chip microcapillary electrophoresis (μCE) system has been developed and applied to the characterization of EVs [9,10]. This system has enabled the sensitive imaging of individual EVs in a dark field by detecting laser light scattered from EVs. Since fluorescence intensity depends on the number of fluorescent molecules bound on the EV surface, low signal to noise ratio (S/N) and photobleaching are serious problems. In contrast, zeta potential does not depend on size but surface charge density of EVs.

3. Biodevice Technology for Exosome Diagnosis

Schematic and photograph of the microfluidic chip-based profiling system for extracellular vesicles are shown in Fig. 1. Briefly, the system comprises a μCE chip, a pair of platinum electrodes, a DC power supply, a 404 nm laser source, a microscope, and a CMOS camera. EVs in microchannels are observed by a laser dark-field microscopy method to detect the motion of small EVs. The laser beam is shaped within 100

μm waist height using cylindrical lens to prevent the background signal generated by scattering at the top and bottom surfaces of the microchannel. Additionally this system is equipped with custom-developed software for adjusting the position of laser beam and objective lens, applying voltage, particle tracking velocimetry and calculating the diameter and zeta potential of EVs.

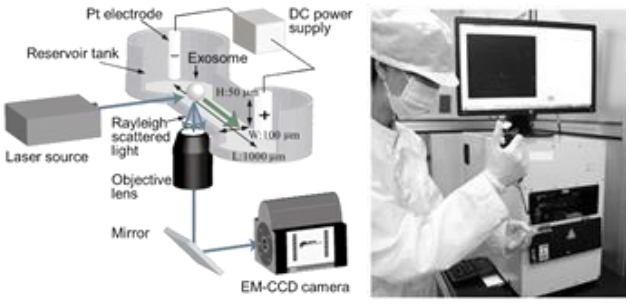

Fig. 1 A schematic diagram and photo of nanoparticle analysis system.

The motion of each EV in microchannel is visualized by a light scattering method and recorded with a computer for particle-tracking analysis. Hydrodynamic diameter and zeta potential of individual EV are calculated from the Brownian motion using the Stokes–Einstein equation and from the electrophoretic motion using the Smoluchowski equation, respectively. Additionally, concentration of EVs can be evaluated from the number of bright spots in the region of imaging. A powerful approach to characterize heterogeneous biological samples is immunoassay method using antibodies for specific molecular recognition. On-chip immune-electrophoresis can provide qualitative and quantitative information on surface molecules of EVs [10]. Since antibody binding increases the amount of positive charges on the EV surface, the immune-reactivity of individual EVs is reflected in their electrophoretic mobility, and hence, their zeta potential.

When on-chip immunoelectrophoresis is performed immediately after the Brownian motion analysis, two-dimensional histograms of the diameter and zeta potential of each EV and exosome of Sk-Br-3; cells can be obtained as shown

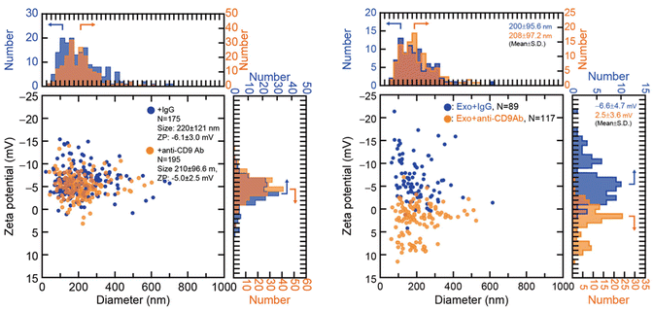

Fig. 2 Simultaneous evaluation of diameter and surface protein expression of individual EVs (left) and exosomes (right) of human breast cancer Sk-Br-3 cells

in Fig. 2. Here, exosomes were purified from EVs by OptiPrep density-gradient ultracentrifugation. There was no significant difference in zeta potential between EVs treated with anti-CD9 antibody and EVs treated with IgG, which was used as an isotype control antibody to estimate the nonspecific adsorption of anti-CD9Ab. In contrast, the zeta potential of exosomes treated with anti-CD9 antibody was different from that of exosomes treated with IgG ($p < 0.05$, t-test).

Moreover, authors' group has also developed an innovative cancer diagnostic device that integrates all the steps of time-consuming blood test on a single cartridge, which comprises separation, purification and sensitive detection of biomarkers in the body fluid such as serum or urine. Therein we have chosen the strategy to use circulating miRNA, stabilized by encapsulation in exosomes, as a promising cancer biomarker (Fig. 3).

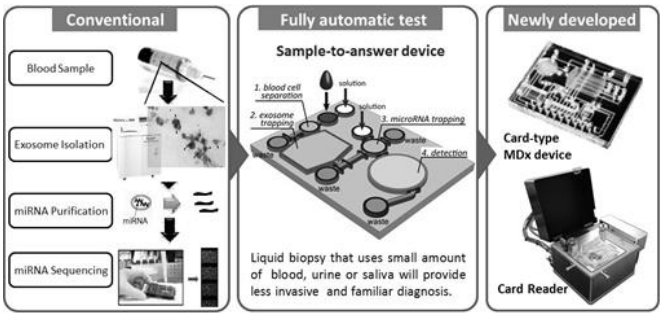

Fig. 3 Cancer diagnosis platform using exosomal miRNA biomarkers is developed using microfluidics and biodevice technology.

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